Biodiversity for sustinable agriculture: Common bean genetic diversity

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Abstract

The immense genetic diversity of genotypes of crops is the most directly useful and economically valuable part of biodiversity. Genetic diversity is a key factor enabling adaptation, and therefore survival, of natural populations in changing environments. And also genetic diversity is essential tool for any breeding program. Leguminous plants, after cereals, include the most economically important species of agricultural interest, considering area cultivated and total production. Among the grain legumes,soybean, peanuts and common beans are the most important commercial crops. Common bean (Phaseolus vulgaris L.) and its related species are important protein sources for the world population. In 2006, the bean industry was valued at $1.2 billion and $180 million in USA and Canada, respectively. The average yield of bean varieties cropped in developing countries is still very low. The analysis of genetic diversity and relationships among different individuals, species, or populations is an important topic in genetics and plant breeding. Since morphological charactersin plants effect from environmental condition, DNA markers provide the most precise tool for measuring genetic relationships, because they are potentially unlimited in number Among the DNA techniques, Amplified Fragment Length Polymorphism (AFLP) is intense and provides a powerful tool for genotype identification, phylogeny The AFLP technique is based on the amplification of short restriction endonuclease digested genomic DNA fragments onto which adaptors have been ligated at both ends.For this purpose common bean genomes were analyzed using AFLP fingerprinting to examine the genetic variation within and among genotypes.. A total of 86 common bean accessions collected from different countries were used in this study. For the AFLP analysis,12 primer combination were used. Acrylamide gels from primer combination were scored according to presence (1) or absence (0) of amplified fragments.The molecular data were analyzed using the NTSYs program. A dendrogram was generated using JMP software (version 3.1, SAS Institute, 1995) based on the UPGMA (unweighted pair-group method of arithmetic average). The eightysix genotypes represented seven different clusters as revealed by AFLP primers. The minimum variation was detected between sample 20, Turkey and sample 24, Turkey (GD = 0.09), and the maximum was found between samples 34 and 28 (GD = 0.80).

Keywords: Biodiversity, Common bean, AFLP

1.INTRODUCTION

Common bean (Phaseolus vulgaris) is an important economic food legume and is widely grown in North, Middle and South America, Eastern Africa, Europe and China. The bean seed is rich in protein, fiber, carbohydrates, minerals and vitamins. Beans provide a good source of protein for rural and urban poor in many developing countries. (Pachico,1989) Common bean originated and was domesticated in the New World and has two major gene pools, The Andean and The Mesoamerican, based on their centers of origin in South and Central America, respectively. (gebts and debouck 1991). Common bean is a diploid (2n=22) legume with a relatively small genome. A few species show an aneuploid reduction to 20 chromosomes. The genome of common bean is one of the smallest in the legume family at 625 Mbp per haploid genome.

DNA markers provide the most precise tool for measuring genetic relationships, because they are potentially unlimited in number and are not affected by the environment (Maciel et al., 2003). During the last two decades, DNA-based molecular markers have been extensively used for a variety of purposes in many animal and plant systems. Among the DNA techniques, amplified fragment length polymorphism (AFLP) is robust and provides a powerful tool for studies of genetic variation, genotype identification, phylogeny (Kafkas 2006), and molecular linkage mapping (Hurtado and Ramstedt 2002). The AFLP analysis provides a higher level of polymorphism than random amplified polymorphism DNA (RAPD) or restriction fragment length polymorphism (RFLP) (Pejic et al., 1998).Amplified fragment length polymorphisms are based on selective and semiquantitative PCR amplification of restriction fragments digested from total genomic DNA. Fragments generated by digestion of DNA with a combination of two restriction endonucleases are linked to suitable adapters and, thereafter, linked DNA fragments are amplified selectively with different primer combinations (Vos et al., 1995). The RFLPs (Becerra-Vela´ squez and Gepts, 1994; Duarte et al., 1999; Metais et al., 2000; Maciel et al., 2001), RAPDs (Haley et al., 1994; Nienhuis et al., 1995; Moura-Duarte et al., 1999; Beebe et al. 2000; Metais et al., 2000), inter simple sequence repeats (ISSRs) (Rosales-Serna et al., 2003), and more recently, AFLPs (Tohme et al., 1996; Caicedo et al., 1999; Maciel et al., 2003; Pallottiniet al., 2004) have been successfully used for the description of diversity in common bean.

In the present paper, AFLP analysis was used to investigate genetic variability at the DNA level in 86 common bean collected from different countries.

2.MATERIAL-METOD

A total of 86 common bean accessions were used in this study (Table 1), including 45 Turkey accessions, 5 Netherlands accessions, 4 Germany accessions,, 3 China accessions, 17 England accessions,11 USA accessions, 1 Bulgaria accessions.

Table 1: A list of 86 P. vulgaris accessions used in AFLP analysis

Genotip Number	Location	Genotip Number	Location
1	Turkey	**20**	Turkey
2	Netherlands	**21**	Turkey
3	Germany	**22**	Turkey
4	Germany	**23**	Turkey
5	Germany	**24**	Turkey
6	Turkey	**25**	Turkey
7	Netherlands	**26**	Turkey
8	Netherlands	**27**	Turkey
9	Netherlands	**28**	USA
10	Turkey	**29**	USA
11	Turkey	**30**	USA
12	Turkey	**31**	England
13	China	**32**	England
14	China	**33**	England
15	Turkey	**34**	England
16	Turkey	**35**	England
17	Turkey	**36**	England
18	Turkey	**37**	England
19	Turkey	**38**	England
39	England	**63**	Turkey
40	England	**64**	Turkey
41	England	**65**	USA
42	Turkey	**66**	England
43	Turkey	**67**	Turkey
44	Turkey	**68**	Turkey
45	Turkey	**69**	Turkey
46	Turkey	**70**	Turkey
47	Turkey	**71**	Turkey
48	Turkey	**72**	Turkey
49	Netherlands	**73**	India
50	USA	**74**	USA
51	USA	**75**	England
52	USA	**76**	England
53	Turkey	**77**	England
54	Turkey	**78**	England

55	Turkey	**79**	England
56	Turkey	**80**	Turkey
57	Bulgaria	**81**	Turkey
58	Turkey	**82**	USA
59	Turkey	**83**	USA
60	China	**84**	USA
61	Turkey	**85**	Turkey
62	Turkey	**86**	Turkey

2.1. DNA extraction

Young leaves from plants collected were harvested and placed in an aluminum foil and kept in liquid nitrogen. Leaf tissue from each individual was ground to a fine powder in liquid nitrogen with a mortar and pestle. Total genomic DNA was extracted following the procedure as described by Doyle & Doyle. The purified DNA was quantified with ND-1000 (Nanodrop, Thermo Co.) spectrophotometer. The DNA quality was also assessed and the concentration determined by visualization under UV light, on 1% agarose gels in TAE (Tris-acetic acid-EDTA) buffer and then agarose gel–stained.

2.2. AFLP analysis

Li-Cor AFLP Kit (catalog number: 830-06195 AFLP 2-DYE Selective Amplification Kit) was used according to the manufacturer's recommendations. According to the kit, 200 ng pure DNA was digested with EcoR I and Mse I restriction enzymes. The enzyme adaptors were ligate to the digested DNA. Selective amplification of restriction fragments was conducted using primers with three selective nucleotide extensions, RD700/800 dyes. Twenty-two primer combinations were used to screen for polymorphism among samples. Amplification products were resolved on 8% acrylamide gel in 1 9 TBE (Tris-borate-EDTA) buffer under 1500 V and 40 mA conditions. Li-Cor 4300s DNA Analyzer machine was used to image, analyze, and screen the bands profile

2.3. Band scoring and data analysis

Each polymorphic AFLP bands were scored manually as present(1) or absent (0) across all 33 genotypes for each primer-paircombination and the values were used to compile binary datamatrix.Onlybright, clearly distinguishable bands were usedin the genetic analysis. Genetic disimilarity estimates were calculated using Jaccard'scoefficient of disimilarity (Jaccard, 1908). JMP software (version 3.1, SAS Institute, 1995) was used to calculate distances and a dendrogram was generated. The accessions were grouped by cluster analysis using the unweighted pair-group method (UPGMA).PIC (polymorphism information content) was calculated from the 1/0 datum matrix. The PIC value refers to the relative value of each marker with respect to the amount of polymorphism it exhibits. PIC was also calculated by 1- Σpi2: , where i= indiviual p and pi = the allele frequencies of the loci. (De Riek,2001).

3.Results and Discussion

3.1.AFLP Marker analysis

The size of bands scored in all the 44 accessions were in the range of 50–450 bp. 86 genotypes were analyzed by AFLP-PCR using 13 selective primer combinations as listed in Table 2. A total of 245 polymorphic bands were generated, and the number of polymorphic bands per each primer combination ranged from 4 (MCAG-EAGG) to 32 (MCAC-EACA) with an average number of 18.8 bands. A representative gel obtained from the primer combination M-CAA/E-ACG (700) is presented in Fig. 1. Polymorphic bands from 86 DNA samples, amplified by 13 AFLP primer combinations, are also listed in Table 2 . The maximum number of polymorphic bands obtained per primer confirmed the high polymorphism determination efficiency of AFLPs in comparison with other marker systems used for common bean such as RAPD (Haley et al., 1994; Maciel et al., 2001; Tiwari et al., 2005) and RFLP (Sonnante et al., 1994; Stockton and Gepts, 2004).

Table 2 Polymorphic bands from 86 DNA samples, amplified by 13 AFLP primer combinations

Primer Number	**Primer Pairs**	**No. of polymorphic bands**
1	MCAC-EACA	32
2	MCAA-EAAC	25
3	MCAA-EACA	15
4	MCTC-EAAG	15
5	MCAG-EACA	27
6	MCAT-EACA	14
7	MCTG-EACA	25
8	MCAC-EAGC	23
9	MCAA-EACG	12
10	MCAA-EAGC	15
11	MCTC-EACT	20
12	MCAG-EAGG	4
13	MCAT-EAGG	18
	TOTAL	245

3.2. Genetic diversity analysis

To determine the genetic relationships among the 86 genotypes, the scoring data (1 for presence and 0 for absence) resulting from the 13 primer combinations were used to compute the dissimilarity matrix according to Jaccard (1908). This dissimilarity matrix was used to generate a dendrogram using the UPGMA method. The 86 genotypes represented seven clades as revealed by AFLP primers

(Fig.2). Group I was the largest one containing 44 accessions that included twenty five Turkey varieties and seven England land races.

As shown in Table 3, the minimum variation was detected between sample 20 Turkey, and sample 24 Turkey (GD= 0,0094) and the maximum was found between sample 34 England and samples 28 USA (GD = 0,80).

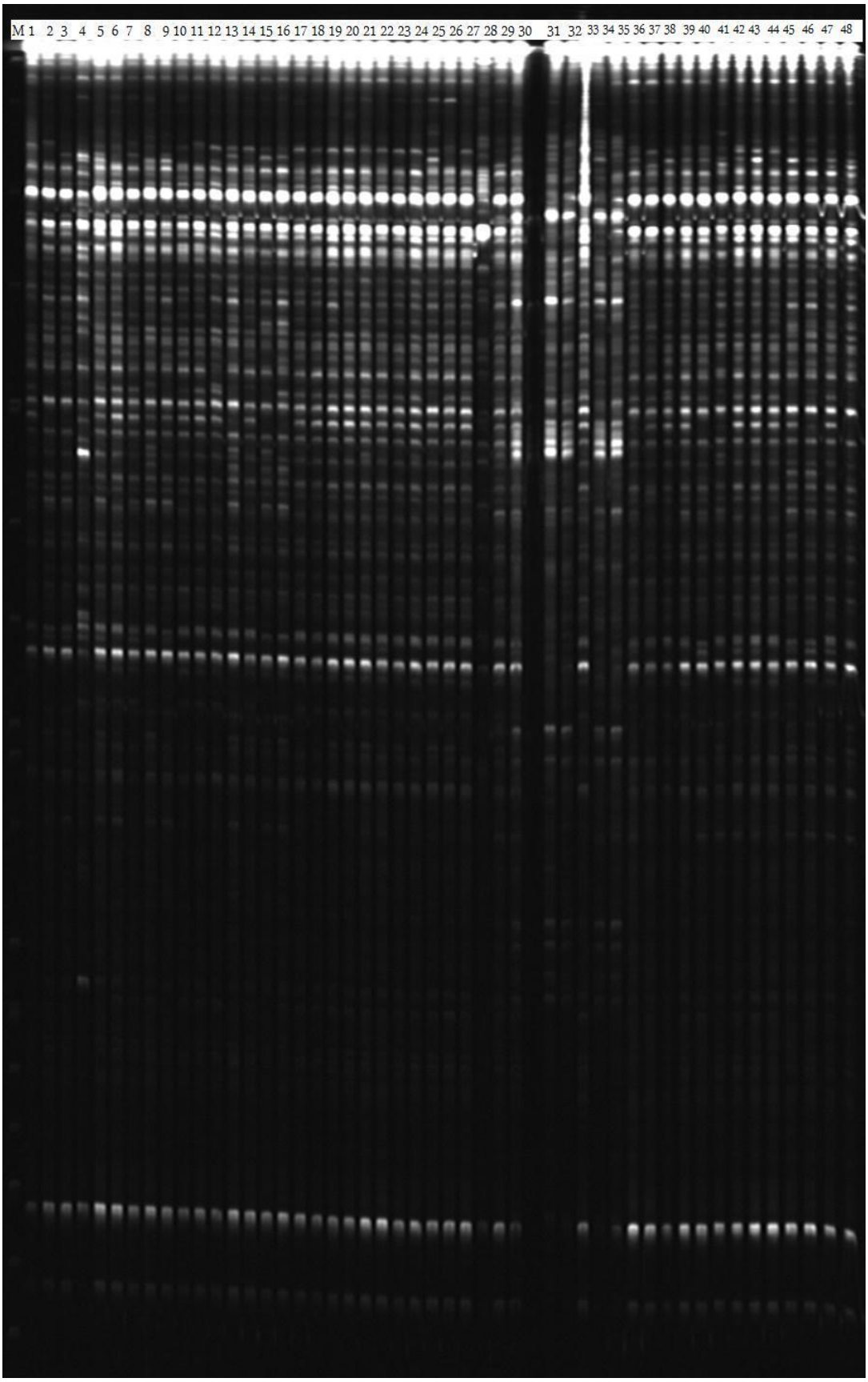

Fig.1. AFLP pattern of 1-48 common bean DNA samples.

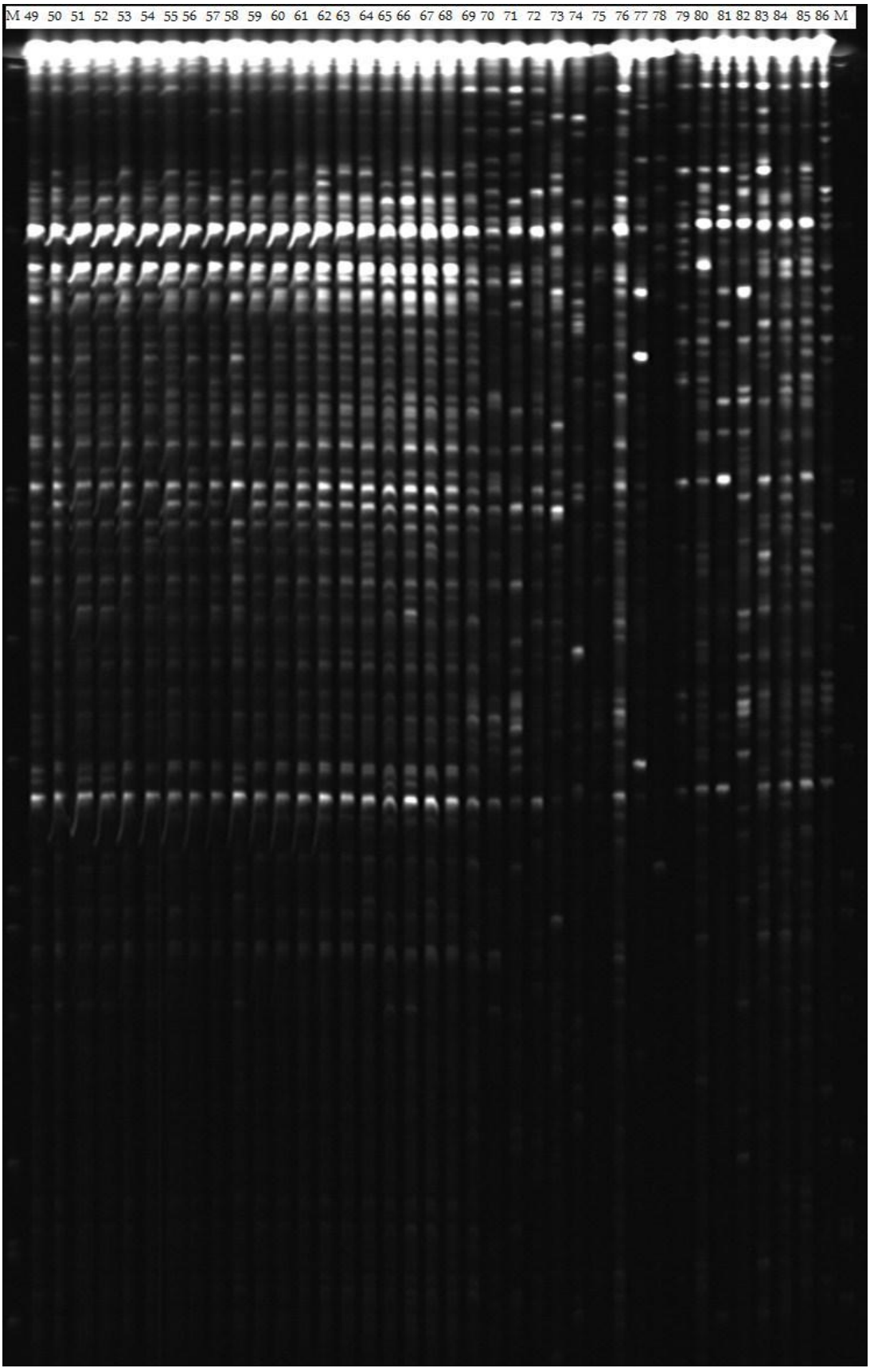

Fig.1. AFLP pattern of 49-86 common bean DNA samples

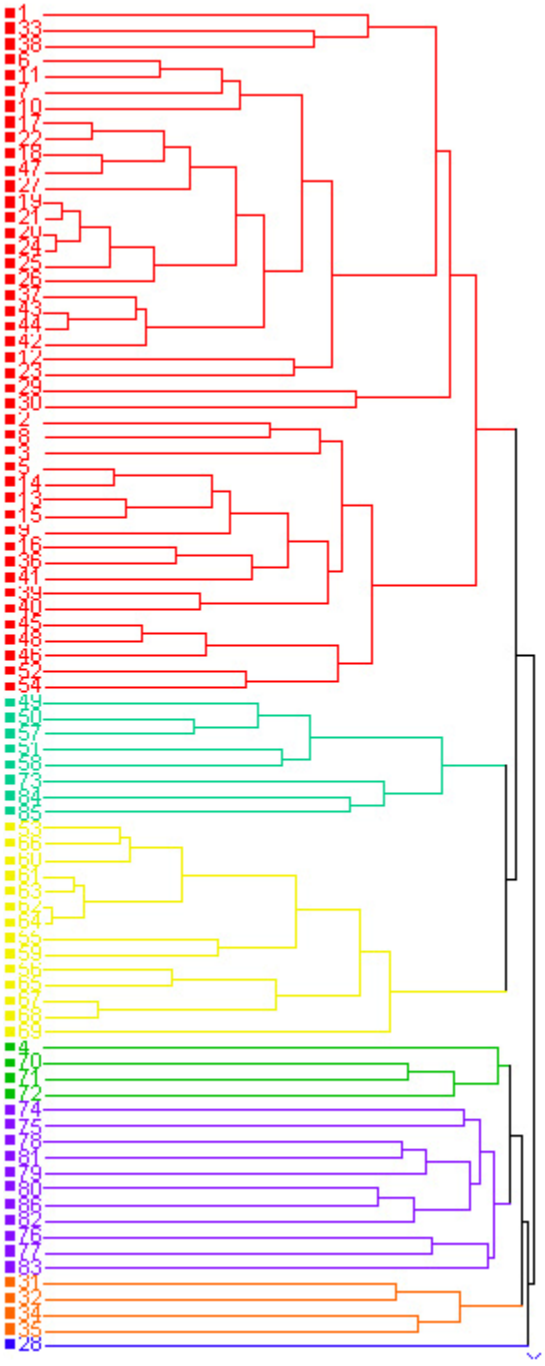

Fig. 2 Dendrogram resulting from UPGMA cluster analysis of 86 common bean genotypes based on data derived from 13 AFLP primer combinations

Studies of genetic diversity using molecular marker and DNA sequencing techniques are necessary if we are to understand a population's genetic structure and phylogeography, identify the center of genetic diversity of a species, and develop effective conservation strategies (Gao, 2003).PCR-based molecular marker techniques play an important role in the analysis of genetic diversity and relatedness for crop plants, where most of the species involved are almost

unknown at the genetic level (Ilgin et al. 2009). In this study, the AFLP method generated large numbers of polymorphic bands. We detected a total of 284 polymorphic bands, and the number of polymorphic bands for each primer combination ranged from from 4 (MCAG-EAGG) to 32 (MCAC-EACA) with an average number of 18.8 bands. Our study shows that AFLP provided a large number of polymorphic bands and a large amount of genotypic information. Grilli Caiola et al. (2004) found the number of polymorphic bands per primer to be 2.01 in their RAPD study.

In conclusion, we have shown that AFLP profiling techniques may provide useful information on the level of polymorphism and diversity in common bean, showing their utility in the characterization of germplasm accessions. AFLP marker systems have comparable accuracy in grouping genotypes of this species according to their gene pool of origin

Örnek No																																									
1	0,00
2	0,21	0,00
3	0,27	0,11	0,00
4	0,42	0,32	0,36	0,00
5	0,24	0,13	0,10	0,30	0,00
6	0,25	0,21	0,17	0,32	0,07	0,00
7	0,24	0,17	0,14	0,33	0,06	0,05	0,00
8	0,21	0,12	0,13	0,32	0,07	0,08	0,07	0,00
9	0,27	0,13	0,10	0,28	0,08	0,13	0,10	0,09	0,00
10	0,18	0,21	0,20	0,34	0,11	0,08	0,09	0,12	0,13	0,00
11	0,24	0,22	0,18	0,30	0,10	0,07	0,10	0,11	0,12	0,07	0,00
12	0,25	0,25	0,17	0,37	0,13	0,10	0,11	0,14	0,13	0,10	0,07	0,00
13	0,24	0,11	0,10	0,33	0,10	0,15	0,14	0,13	0,08	0,13	0,12	0,13	0,00
14	0,29	0,13	0,10	0,28	0,04	0,09	0,08	0,09	0,06	0,11	0,10	0,11	0,08	0,00
15	0,25	0,10	0,05	0,32	0,05	0,12	0,09	0,10	0,07	0,14	0,13	0,14	0,05	0,05	0,00
16	0,30	0,12	0,09	0,34	0,09	0,14	0,11	0,14	0,09	0,18	0,15	0,18	0,09	0,09	0,04	0,00
17	0,28	0,18	0,15	0,34	0,07	0,06	0,07	0,10	0,13	0,08	0,09	0,08	0,13	0,07	0,10	0,12	0,00
18	0,25	0,21	0,15	0,32	0,09	0,06	0,07	0,10	0,11	0,06	0,07	0,06	0,13	0,07	0,10	0,14	0,04	0,00
19	0,27	0,17	0,12	0,30	0,06	0,05	0,06	0,09	0,10	0,07	0,06	0,07	0,10	0,04	0,07	0,11	0,03	0,03	0,00
20	0,24	0,17	0,14	0,33	0,06	0,05	0,06	0,09	0,12	0,05	0,08	0,09	0,12	0,06	0,09	0,13	0,03	0,03	0,02	0,00
21	0,24	0,15	0,12	0,33	0,04	0,05	0,04	0,07	0,10	0,07	0,08	0,09	0,10	0,06	0,07	0,11	0,03	0,05	0,02	0,02	0,00
22	0,27	0,15	0,12	0,33	0,04	0,07	0,06	0,09	0,10	0,09	0,10	0,09	0,12	0,04	0,07	0,11	0,03	0,05	0,04	0,04	0,04	0,00
23	0,28	0,23	0,15	0,34	0,13	0,10	0,13	0,16	0,15	0,14	0,11	0,10	0,17	0,11	0,14	0,18	0,10	0,10	0,07	0,09	0,09	0,11	0,00
24	0,23	0,18	0,15	0,34	0,07	0,06	0,07	0,10	0,13	0,06	0,09	0,10	0,13	0,07	0,10	0,14	0,04	0,04	0,03	0,01	0,03	0,05	0,08	0,00
25	0,25	0,18	0,15	0,32	0,09	0,08	0,09	0,12	0,13	0,08	0,07	0,10	0,11	0,07	0,10	0,14	0,06	0,06	0,03	0,05	0,05	0,07	0,08	0,04	0,00
26	0,25	0,18	0,17	0,34	0,09	0,08	0,09	0,12	0,15	0,10	0,07	0,10	0,15	0,09	0,12	0,14	0,06	0,08	0,05	0,05	0,05	0,07	0,06	0,04	0,06	0,00
27	0,28	0,18	0,15	0,34	0,09	0,08	0,09	0,10	0,13	0,10	0,09	0,08	0,13	0,07	0,10	0,14	0,04	0,04	0,03	0,05	0,05	0,05	0,10	0,06	0,06	0,08	0,00
28	0,61	0,42	0,53	0,50	0,53	0,55	0,50	0,55	0,53	0,58	0,56	0,58	0,50	0,50	0,48	0,48	0,51	0,55	0,53	0,53	0,53	0,50	0,55	0,51	0,51	0,48	0,51	0,00
29	0,37	0,29	0,25	0,44	0,18	0,20	0,18	0,22	0,23	0,24	0,21	0,20	0,25	0,18	0,20	0,24	0,17	0,17	0,16	0,18	0,16	0,16	0,17	0,17	0,17	0,17	0,15	0,50	0,00
30	0,40	0,24	0,18	0,38	0,12	0,20	0,16	0,20	0,16	0,22	0,18	0,20	0,18	0,12	0,13	0,15	0,15	0,15	0,14	0,16	0,14	0,12	0,22	0,17	0,17	0,17	0,13	0,56	0,14	0,00
31	0,45	0,42	0,44	0,53	0,41	0,42	0,41	0,42	0,47	0,40	0,41	0,45	0,44	0,41	0,40	0,42	0,40	0,42	0,38	0,38	0,38	0,38	0,48	0,40	0,37	0,40	0,42	0,63	0,47	0,38	0,00
32	0,41	0,38	0,40	0,45	0,37	0,36	0,34	0,38	0,42	0,36	0,37	0,44	0,40	0,37	0,36	0,36	0,36	0,38	0,34	0,34	0,34	0,34	0,44	0,36	0,33	0,36	0,38	0,55	0,40	0,34	0,13	0,00
33	0,23	0,25	0,22	0,42	0,17	0,14	0,15	0,16	0,20	0,14	0,17	0,16	0,17	0,17	0,16	0,21	0,14	0,12	0,13	0,11	0,13	0,15	0,21	0,12	0,14	0,16	0,16	0,58	0,29	0,29	0,42	0,38	0,00
34	0,56	0,56	0,55	0,55	0,51	0,53	0,51	0,53	0,55	0,50	0,51	0,56	0,58	0,51	0,53	0,56	0,50	0,53	0,48	0,48	0,48	0,48	0,53	0,50	0,47	0,50	0,50	0,80	0,55	0,42	0,20	0,21	0,53	0,00
35	0,45	0,40	0,36	0,44	0,38	0,40	0,38	0,40	0,44	0,40	0,36	0,37	0,41	0,38	0,37	0,40	0,37	0,40	0,36	0,36	0,36	0,36	0,40	0,37	0,34	0,34	0,40	0,53	0,41	0,33	0,18	0,17	0,40	0,22	0,00
36	0,28	0,12	0,11	0,32	0,07	0,12	0,09	0,12	0,09	0,16	0,17	0,18	0,11	0,09	0,06	0,06	0,12	0,14	0,11	0,11	0,09	0,09	0,18	0,12	0,14	0,14	0,14	0,45	0,20	0,15	0,40	0,30	0,18	0,53	0,37	0,00
37	0,24	0,15	0,12	0,36	0,06	0,07	0,06	0,07	0,10	0,09	0,10	0,11	0,10	0,08	0,07	0,11	0,05	0,07	0,04	0,04	0,02	0,06	0,11	0,05	0,07	0,07	0,07	0,53	0,16	0,16	0,38	0,34	0,13	0,48	0,36	0,09	0,00
38	0,23	0,21	0,17	0,34	0,09	0,08	0,11	0,10	0,15	0,10	0,11	0,14	0,15	0,11	0,12	0,16	0,08	0,08	0,07	0,05	0,07	0,09	0,14	0,06	0,10	0,10	0,10	0,55	0,22	0,20	0,40	0,36	0,12	0,50	0,37	0,12	0,07	0,00
39	0,24	0,15	0,14	0,33	0,06	0,09	0,06	0,09	0,10	0,09	0,12	0,13	0,14	0,08	0,09	0,11	0,07	0,09	0,06	0,06	0,04	0,06	0,13	0,07	0,09	0,09	0,09	0,53	0,18	0,16	0,41	0,37	0,17	0,51	0,38	0,09	0,06	0,11	0,00
40	0,27	0,11	0,10	0,36	0,06	0,11	0,08	0,07	0,08	0,13	0,14	0,15	0,08	0,06	0,05	0,09	0,09	0,11	0,08	0,08	0,06	0,06	0,15	0,09	0,11	0,11	0,11	0,50	0,21	0,16	0,41	0,37	0,17	0,51	0,38	0,09	0,06	0,11	0,06	0,00
41	0,22	0,11	0,12	0,36	0,08	0,15	0,12	0,13	0,12	0,15	0,14	0,17	0,12	0,10	0,09	0,11	0,13	0,15	0,12	0,12	0,10	0,10	0,17	0,13	0,15	0,11	0,13	0,56	0,23	0,16	0,41	0,34	0,22	0,55	0,38	0,09	0,12	0,17	0,12	0,12	0,00
42	0,20	0,15	0,16	0,38	0,10	0,11	0,10	0,09	0,12	0,07	0,08	0,13	0,10	0,12	0,11	0,13	0,09	0,09	0,08	0,06	0,06	0,10	0,15	0,07	0,09	0,09	0,09	0,56	0,23	0,18	0,38	0,34	0,15	0,48	0,36	0,13	0,06	0,09	0,10	0,10	0,10	0,00

Table 3 Genetic distance matrix computed according to Jaccard (1908)'s coefficient based on AFLP data

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